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Microalgae bioengineering: From CO_2 fixation to biofuel production

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ABSTRACT

The recognised deficiencies in sustainable development and the extensive environmental deterioration and global warming concerns caused by anthropological CO_2 emissions are major issues facing the world today. Massive reduction in atmospheric CO_2 concentration, through the development of processes that utilize CO_2 or minimise CO_2 emissions, is critical to ensure environmental sustainability. One of the major contributors to anthropological CO_2 emission is the combustion of petroleum fuels in vehicular engines for transportation. Biofuel, as an alternative to petroleum transport fuels, has become a partial substitute for fossil fuel. The use of microalgae for biofuel production has gained enormous research interests in recent years, primarily due to the ability to photosynthetically convert CO_2 (a biology-inspired process engineering route) into potential biofuel biomass, as well as food, feed stocks, and high value biochemicals. In this review, the CO_2 fixation ability of microalgae in comparison to other plant species and genetic engineering methods of improving microalgae photosynthetic rate, have been discussed. Advances in bioprocess technologies for microalgal biomass creation and biodiesel production are also described and other important matters are discussed.

Contents

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Table 1
Comparing CO_2 capturing capacity and biodiesel productivity of microalgae and higher plants.

Plants	CO_2 capture capacity (%)[a]	Productivity (g/m^2 d)	Productivity of the theoretical max (%)	Lipid content of biomass (%)	Biodiesel productivity (g/m^2 d)[b]
Theoretical [8]	100	77	100	–	N/A
Green microalgae [9]	26–52	20–40	27–54	30–45	6–12
Chlorella [10,11]	22–26	17–20	22–27	40–60	5.4–6
Miscanthus giganteus [8,12,13]	10–22	8–17	11–22	N/A	2.4–5.4
Oilseed rape [14]	1–2	0.8–1.6 (of seed)	1.0–1.2 (of seed)	40–44 (of seed)	0.24–0.48
Soya [15]	N/A	0.65 (of seed)	0.8 (of seed)	20 (of seed)	0.2
Jatropha [16]	N/A	2.2 (of seed)	2.8 (of seed)	30 (of seed)	0.66

[a] In the theoretical view, to produce 77 g biomass, 3.6 mol (158.4 g) of CO_2 is captured by autophyte [8].
[b] It is assumed that 30% of biomass is lipid [17].

1. Introduction

In recent years, due to increasing awareness of global energy crisis in conjunction with escalating concerns regarding sustainability and the environment, interest in biofuels and its production has fast risen [1]. The increase in atmospheric CO_2 concentration, which is the main component of greenhouse gas emissions, poses great challenges to worldwide pro-environment and sustainability. Current available technologies for CO_2 capture include physicochemical adsorption, injection into deep oceans or geological formations, and enhanced biological fixation. Physicochemical adsorption process is difficult to control, and the adsorbent materials are typically non-renewable and expensive. Abiotic methods, such as direct injection of CO_2 into the deep ocean, geological strata, old coal mines, oil wells or saline aquifers, as well as mineral carbonation of CO_2, present significant challenges of high space requirements and potential leakage with time [2]. One of the most environmentally sustainable ways to reduce greenhouse gas emissions associated with energy production is to generate energy from reduced-carbon-emission sources. With the progress of research and development into new energy forms, biofuel is thought of as an effective and practical alternative transport fuel that may, in the future, play a significant role in the reduction of transportation related CO_2 emissions.

CO_2 biological fixation is a long-term environmental sustainable technology. CO_2 in the atmosphere and flue gas is converted into biomass by autotrophs, whilst nutrient utilization and energy feedstock production is achieved in a sustained fashion [3]. Different kinds of energy compounds such as oil, ethanol, and bio-hydrogen can be generated from higher plants, photosynthetic bacteria and microalgae.

Compared to other plant feedstock, microalgae have a number of advantages in CO_2 capture and bio-oil generation. These include (i) high photosynthetic conversion efficiencies, (ii) rapid biomass production rates, (iii) the capacity to produce a wide variety of biofuel feedstock, (iv) ability to thrive in diverse ecosystems, (v) distinguished environmental bioremediation such as CO_2 fixation from the atmosphere or flue gas, and water purification [4,5], (vi) non competitiveness for land with crops and (vii) non competitiveness with the food market. Unlike plants, unicellular microalgae do not partition large amounts of biomass into supportive structures such as stems and roots that are energetically expensive to produce and often difficult to harvest and process for biofuel production. Additionally, microalgae have carbon concentrating mechanisms that suppress photorespiration [6,7]. The CO_2 capturing capacity and oil productivity of different kinds of plants are summarized by the authors in Table 1. The process merit of CO_2 fixation by microalgae cultivation is that the biomass produced can be converted efficiently into biofuels for energy production directly.

Microalgal biodiesel production system involves the following process steps: cultivation, harvesting, dewatering, extraction, and transesterification [18]. To achieve high oil yields and CO_2 fixation capacity during cultivation, the key process considerations are the choice of microalgal strain, cultivation conditions, and the cultivation system (photobioreactors or open ponds). Different technologies are available for harvesting, dewatering, extraction, and transesterification. However, high efficiency, energy saving and low CO_2 emission technologies are the optimum targets for full-scale industrial application of microalgae biotechnology.

2. Microalgae genetic engineering

In recent years, new biotechnological approaches relating to genome perturbation of microalgal cells to endow them with different properties are rapidly increasing. However, the full potential of genetic engineering of some microalgal species, particularly diploid diatoms, can be fully realized only if conventional breeding methods become firmly established, thereby allowing useful mutations to be easily combined [19]. Significant advances in microalgal genomics have been achieved during the last decade [19–21]. Expressed sequence tag databases have been established; nuclear, mitochondrial, and chloroplast genomes of several microalgae strains have been sequenced. Historically, the green algae *Chlamydomonas reinhardtii* has been the focus of molecular and genetic phycological research. Therefore, most of the tools developed for the expression of transgenes and gene knockdown are specific for this kind of species. Current genetic engineering pursuits are towards microalgae that are of greater interest in industrial applications and environmental conservation [19]. To improve microalgae biomass or lipid production and CO_2 capturing efficiency, several approaches have been developed.

2.1. Genetic engineering of microalgae to enhance carbohydrate and protein storage

Starch is the basic energy storing biochemical of plants including microalgae. Adenosine diphosphate-glucose pyrophosphorylase (AGPase) and 3-phosphoglyceric acid (3-PGA) are the rate-limiting molecules of starch synthesis [19,22,23]. Much has been reported on the catalytic and allosteric properties of AGPases in crop plants to increase starch production [24–26]. Most cellular AGPases are far from the pyrenoid and this could result in 3-PGA inactivation, some AGPases, such as Mos(1-198)/SH2 AGPase, have activity even without an activator [27]. In some microalgal species, starch production is improved when Mos(1-198)/SH2 AGPase or other AGPases is overexpressed. Although, the precise mechanism of starch catabolism in most microalgae is largely unknown, some studies have reported decreasing starch degradation in microalgae cells [28,29]. In *A. thaliana*, α-amylase is thought to participate in starch degradation. Interestingly, starch is degraded even when all amylases are knocked out, indicating phosphorolytic mechanism for starch degradation [30,31]. Catabolism of starch provides stock and intermediate of lipid and protein synthesis, and this is sometimes the key rate-limiting step of lipid and protein synthesis [31].

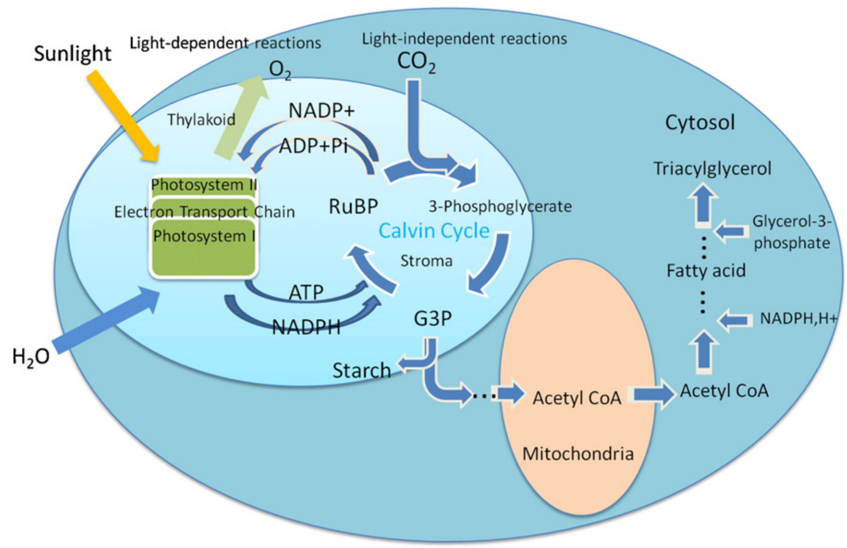


Fig. 1. Schematics of photosynthesis, CO_2 fixation and carbon accumulation in microalgae cells.

One of the main biochemicals in microalgae is protein. Protein synthesis is the most important and complicated in all cells. The process of protein synthesis mainly consists of amino acid synthesis, peptide chain condensation reaction (transcription and translation) and modification of the primary protein. Amino acid synthesis rate can be adjusted by changes in the expression levels of the enzymes involved. The rate of synthesis of the enzymes involved can be altered by changing the activities of the related gene [32]. For example, when a synthetic amino acid production rate is too high, the pathway of the enzyme gene can be suppressed. However, the encoding gene can be disinhibited in case the synthetic amino acid concentration declines, thus the expression of enzyme required for amino acid synthesis increases. Some work has been performed in knocking out relevant gene segments involved and this resulted not only in altering protein storage, but also some cell functions with deleterious effects on cellular growth and proliferation [33,34].

2.2. Genetic engineering of microalgae to enhance lipid storage

Many microalgae do not produce large amounts of lipids during logarithmic growth. Instead, when they encounter environmental stress, such as a lack of nitrogen, they slow down their rate of proliferation and start producing energy storage products, such as lipids and starch [35]. It will be interesting to see how overexpression of genes, which control the lipid synthesis pathway, affect microalgal proliferation. It may be that increasing rate of lipid synthesis could result in cell division reduction. In this case, overexpression of lipid synthesis genes may still be beneficial if they can be controlled by an inducible promoter that can be activated once the microalgal cells have reached high densities and have entered the stationary phase. For example in *C. reinhardtii*, copper-responsive elements are inducible promoters [36,37], and in diatoms nitrate-responsive species are inducible promoters [38]. Inhibiting lipid catabolism may also cause problems with proliferation and biomass productivity since microalgae often rely on catabolic pathways to provide energy and precursors for cell division [19]. Some efforts have been made to improve the expression of enzymes that are involved in the pathways of fatty acid synthesis [34]. One initial step in fatty acid synthesis is the conversion of acetyl-coenzyme A (CoA) to malonyl-CoA, catalyzed by acetyl-CoA carboxylase (ACCase), and this is considered as the first committed step in fatty acid biosynthesis in many organisms [39]. It has been shown that ACCases overexpression does not increase lipid production significantly in *C. cryptic* and *A. thaliana* [40,41], but interestingly increase up to 5-fold of triacylglycerol (TAG) content in potato tubers. The reason may be that ACCase level is a limiting step in lipid biosynthesis mainly in cells which normally do not store large amounts of lipid [19]. With regards to increasing the total amount of seed oils, some fantastic results have been achieved through overexpression of genes involved in TAG assembly. For example, overexpression of a glycerol-3-phosphate dehydrogenase (G3PDH) in *B. napus* resulted in 40% increase in the lipid content of the seeds [42]. G3PDH catalyzes the formation of glycerol-3-phosphate, and this suggests that genes involved in TAG assembly are important for total seed oil production. This result is further supported by several other studies in which overexpression of TAG assembly genes result in increasing seed oil content [43–45].

Another strategy to increase lipid accumulation is to decrease lipid catabolism. In the lipid catabolism process, acyl-CoA oxidase, acyl CoA synthase, carnitine acyltransferase I, fatty acyl CoA dehydrogenase are the key enzymes of β-oxidation of fatty acids. Research work focused on knocking out some of these enzyme genes has been reported to increase lipid storage [46,47]. Microalgae initiate TAG storage during light cycles and use it during dark cycles to provide cellular energy for cell growth and proliferation. Consequently, inhibition of β-oxidation would prevent the loss of TAG during the dark cycles, but also possibly at the cost of reduced growth rate. Several publications have shown that knocking out genes involved in *S. cerevisiae* β-oxidation does not only lead to increased amounts of intracellular free fatty acids, but also extracellular fatty acid secretion [48,49]. However, due to the fact that cells rely on β-oxidation of fatty acids for cellular energy under certain physiological conditions, this also might have deleterious effects on cellular growth and proliferation.

2.3. Genetic engineering of microalgae to improve photosynthetic efficiency

Microalgae are considered as a great model of photosynthetic efficiency organisms. As the source of energy, light plays a vital role in all plant growth. For microalgae, optimal light delivery affects the photosynthetic efficiency directly [50]. Irrespective of the method used to cultivate the microalgal biomass, the photosynthetic apparatus must consist of photo systems (summarized in Fig. 1 by the authors), where light energy is used for photochemical reactions,

Table 2
Nutrient functions during microalgae cultivation.

Nutrition	Main ingredients	Function	Suitable content range [58–61]
Carbon source	CO_2, HCO_3^-, CO_3^{2-}, etc.	Provide C to the hole cell, etc.	1–10 g/L
Nitrogen source	NO_3^-, Urea, AA, N_2, etc.	Provide N to the hole cell, etc.	10–2000 mg/L
Phosphorus	Hydrophosphate, phosphate, etc.	Provide P to every reaction in cells, etc.	10–500 mg/L
Sulphur	sulphate etc.	Provide S to proteins and reactions, etc.	1–200 mg/L
Inorganic salts	K, Ca, Na, Mg, etc.	Maintain cell structure and activity, etc.	0.1–100 mg/L
Trace elements	Fe, Zn, Mn, Pb, Cd, etc.	Be coenzyme factors, etc.	0.01–10 mg/L
Vitamin	V_B, V_C, V_E, etc.	Aid to cell division, etc.	0.01–1000 μg/L

surrounded by antenna in the chloroplast complexes that harvest the light energy and transport to the photo systems. Microalgae have evolved to absorb more light than needed for their photosynthetic requirements. The excess light energy is dissipated as heat and fluorescence [51]:

$$CO_2 + H_2O + 9.5hv \rightarrow \frac{1}{6}C_6H_{12}O_6 + O_2 \quad (1)$$

According to Eq. (1), when photon utilization increases in microalgae, light conversion efficiency increases, and the ability of CO_2 fixation increases as well [8]. Several attempts have been made to improve the photosynthetic efficiency and reduce the effects of photoinhibition on microalgal growth. Most of these are focused on reducing the size of the chlorophyll antenna [19,52], and this can be achieved by the following means.

On a macro-scale, microalgae growing at high light intensity is kept for a long period to induce mutagenesis in which the antenna size decreases, although the change is readily reversible when the cells are subsequently transferred to light with low intensity [53]. Moreover, at high light intensity levels there is less efficient use of absorbed light energy, and also biochemical damage to the photosynthetic machinery (photoinhibition), making light energy utilization even less efficient. Thus the highest photosynthetic efficiency is realized at a low light intensity [50]. This approach may seem counterintuitive, but it has two positive effects. It permits a higher light penetration in high-density cultures, and also allows a higher maximum rate of photosynthesis due to the fact that cells are less likely to be subjected to photoinhibition since their light-harvesting complexes absorb less light [8]. Manipulation of antenna size through nutrient levels has received less attention. However, conditions of nutrient deprivation leading to increased productivity of TAGs may cause a reduction in chlorophyll levels per cell [54], suggesting that nutrient deprivation may have multiple beneficial consequences for feedstock production.

On a micro-scale, genetic manipulations could be employed to modify the gene sequences involved. Earlier research relied on random mutagenesis strategies to generate mutants with fewer or smaller chlorophyll antenna, but recent publications efficiently used an RNAi-based strategy to knock down both LHCI and LHCII in *C. reinhardtii* [55,56]. This strategy can most likely be applied to many different microalgae more easily than a random mutagenesis approach. It is quite obvious that manipulation of light-harvesting complexes can lead to increased biomass productivity under high light in controlled laboratory conditions. However, it remains to be seen how well these mutants will perform in larger scale cultures with more varied conditions and perhaps with competition from wild invasive microalgal or bacteria species.

3. Microalgae cultivation for biomass production and CO_2 capture

Significantly, microalgal cultivation systems can be tightly controlled and optimized. Temperature, pH, and nutrient and CO_2 concentrations can be monitored and optimized for maximum biomass and oil yields [57]. Light, CO_2 and inorganic salts are indispensable. Microalgae convert the sunlight energy into chemical energy. Simultaneously, CO_2 is fixed and transferred to carbon-containing compounds, such as carbohydrates, lipids and proteins. Therefore, the CO_2 fixation capacity reflects in the microalgae biomass.

3.1. Growth medium

The growth medium must provide sufficient nutrients for microalgae growth. As shown in Table 2 summarized by the authors, elements such as carbon, nitrogen, phosphorus, and sulphur are the most important elements constituting the microalgal cells. Other essential inorganic salts include iron, magnesium, trace elements, and in some cases, silicon [62]. It is important to develop a balanced medium for optimum microalgae cultivation and CO_2 fixation [5,63]. For different products accumulation by microalgae, different formulations are required. Lipids are the most desirable component for biodiesel; whilst, high value health care products rely mostly on proteins and some fatty acid forms. Cells with higher lipid contents and lower carbohydrates and proteins have elevated calorific values and produce higher yields of oil when processed via biomass liquefaction [64–66].

Nitrogen limitation has been observed to result in lipid content increase in many chlorella strains such as *Chlorella emersonii* (63%), *Chlorella minutissima* (56%), *Chlorella vulgaris* (57.9%), *Chlorella luteoviridis* (28.8%), *Chlorella capsulata* (11.4%), and *Chlorella pyrenoidosa* (29.2%) [67]. It has been reported that, under nitrogen deficient conditions, *Neochloris oleoabundans* cells accumulate 35–54% lipids with 80% triglycerides component [66]. It has also been reported that the triglycerides accumulated in *Nannochloris* sp. under nitrogen deficient conditions could be 2.2 times of that in nitrogen sufficient cultures [68].

3.2. Photon requirement

Sunlight is the main source of energy for microalgae. Although light intensity requirements of typical microalgal cells are relatively low compared to those of higher plants, microalgal metabolic activity rates usually increases with increasing light intensity up to 400 mmol m^2 s^{-1} [69]. For example, the saturating light intensity of Chlorella and *Scenedesmus* sp. is 200 mmol m^2 s^{-1} [3]. The thermophilic *Chlorogleopsis* sp. exhibits high light adaptability, growing successfully under both high light intensity (246.1 mmol m^2 s^{-1}) and low light intensity (36.9 mmol m^2 s^{-1}) with the optimum light intensity 200 mmol m^2 s^{-1} [70]. Under low light conditions, many microalgal species switch from phototrophic to heterotrophic growth, and some can even grow mixotrophically. As discussed earlier, the optimum light intensity of microalgae could be modified genetically and altering the size of the chlorophyll antenna. These strategies may not be the best for microalgae cultivation in outdoor open ponds, but may be valid to increase biomass production for microalgae grown in photobioreactors with atmospheric or flue gas CO_2 under a continuous or periodic light provision.

3.3. CO_2 fixation and storage

Inside the chloroplast, CO_2 can be fixed by rubisco to produce two molecules of 3-phosphoglycerate [51]. Through a series of reactions these two 3-carbon organic acids are synthetized as substrates for starch and oil production. However, oxygen can compete with CO_2 for Calvin Cycle by rubisco. Products of the oxygenase reaction are 3-phosphoglycerate and 2-phosphoglycolate. 2-Phosphoglycolate is subsequently metabolized to glycine, which, when condensed with another glycine molecule to produce serine, results in the loss of CO_2 [51]. This carbon loss diminishes the ability of the Calvin cycle to regenerate the five-carbon sugar substrate ribulose bisphosphate-required for Calvin cycle by rubisco, and further reducing the efficiency of photosynthesis. This overall process is known as photorespiration because it occurs largely in the presence of light [71]. The process of photorespiration reduces photosynthetic carbon fixation efficiency by 20–30% [72]. To reduce the competitive inhibition of oxygen on Calvin cycle by rubisco, microalgae actively pump and store sufficient CO_2 to elevate internal CO_2 concentrations above equilibrium levels with air [73]. Furthermore, under normal growth conditions of some species, glycolate formation and photorespiration are absent [74].

One of the most attractive features of microalgal biomass production is the potential to fix CO_2 from the atmosphere or combustion flue gas (contains 5–30% CO_2 [75]). Between 1.6 and 2 g of CO_2 is captured for every gram of microalgal biomass produced [76]. *Chlorococcum littorale* shows an exceptional tolerance to high CO_2 concentrations of up to 40% (v/v) [77]. It has also been reported that *Scenedesmus obliquus* and *Spirulina* sp. show good capacities to fix CO_2 when cultivated at 30 °C in a temperature-controlled three-stage serial tubular photobioreactor [78]. For *Spirulina* sp., the maximum specific growth rate and maximum productivity were 0.44 per day and 0.22 g L^{-1} per day, with both 6% and 12% CO_2, respectively, whilst the maximum cell concentration was 3.50 g L^{-1} dry cell weight. Two green microalgal strains, *Chlorella* sp. UK001 and *C. littorale*, selected by Murakami and Ikenouchi [79], showed high CO_2 fixation rates exceeding 1 g L^{-1} CO_2 per day. *Botryococcus braunii* SI-30, which showed the ability to produce high hydrocarbon concentrations, was recommended as a promising candidate for combined CO_2 mitigation and biofuel production [5]. *S. obliquus* SJTU-3 and *C. pyrenoidosa* SJTU-2 showed high CO_2 levels (30–50%) to be favourable for high accumulation of total lipids and polyunsaturated fatty acids [80]. Fortuitously, the benefits of flue gas injection on Microalgal growth were observed to be greater than the growth impacts solely attributed to inhibition of photorespiration by high CO_2 concentrations, with 30% increase in biomass productivity. This was attributed to the presence of nutrient (sulphur and nitrate) in the flue gas [81].

3.4. Temperature and pH

Temperature is one of the major factors that regulate cellular, morphological and physiological responses of microalgae. Higher temperatures generally accelerate the metabolic rates of microalgae, whereas low temperatures lead to inhibition of microalgal growth [69]. In suitable temperature condition, the enzymes in microalgal cells possess the highest activity. Different species have different optimal temperatures. However, optimal temperatures are also influenced by other environmental parameters, such as light intensity. For many species, optimal growth temperatures of 25–35 °C have been reported, with maximum cell densities obtained around 30 °C [3,82].

Most microalgal species are favoured by neutral pH. However, some species have optimum growths under acidic or alkaline conditions. For example, the optimal pH of *Spirulina platensis* [83] and *C. littorale* [84] are around 9 and 4, respectively. The concentration of CO_2 or $CO_3{}^{2-}$ has a strong impact on pH value in microalgal bioreactor systems. In the cultivation of *S. platensis*, increasing CO_2 can lead to higher biomass productivity, whilst decreasing pH can have an adverse effect on microalgal physiology. CO_2 uptake by microalgal culture results in increasing pH. Similarly, the speciation of NH_3 and $NH_4{}^+$ in microalgal culture systems is strongly dependent on pH-NH_3 electron transport in the microalgal photosystem and competes with water molecules in oxidation reactions, thus leading to release of O_2 [83]. In some cases, pH is controlled by adjusting CO_2 and $NH_4{}^+$ concentrations.

3.5. Cultivation strategies

Microalgae cultivation strategy also plays an important role in CO_2 capture and biomass accumulation. Some researchers have focused on heterotrophic cultivation for higher biomass productivities [85–88]. In heterotrophic cultivation, microalgae consume organic carbon and nitrogen sources, release much CO_2 which is the main component of greenhouse gases. On the other hand, the economics is very high due to carbon/nitrogen sources and energy input. Therefore, autotrophic cultivation strategy is used in large-scale commercial microalgal biomass cultivation systems.

The two major classes of microalgae growth systems, ponds and closed photobioreactors, have different advantages. Open ponds of large area are relatively cheaper to build, and easy to operate, but there are always issues with contamination, difficulty of maintaining constant cultivation conditions, especially temperature, low cell densities, and substantial harvesting costs. To avoid contamination, highly selective conditions, such as high saline media or at high culture pH, have been used in some cases to guarantee dominance by the selected strain, even though such conditions are not available for all species. Due to these drawbacks, much attention has been paid to closed photobioreactors particularly for biomass production [50]. However, prior to the analysis of specific photobioreactor configurations, general design considerations are essential to evaluate and compare different bioreactor designs effectively [89]. Vertical, flat plate, annular, plastic bags, and air lifted glass or plastic tubular reactors have been in focus [65,90,91]. Contamination can be avoided in closed photobioreactors if operated under aseptic conditions at an expense. In terms of energy consumption, closed photobioreactors typically require energy for pumping and sparging, and have much embodied energy in the materials of construction, although this might be offset by the higher productivity in closed systems [50]. Photobioreactor system has been suggested for the production of high-value long-chain fatty acids or proteins, and open pond system is perhaps more suitable for cultivating microalgae for biofuels [92–94]. Nevertheless, CO_2 can be efficiently fixed autotrophically, by both closed photobioreactors and open pond systems. Furthermore, some new techniques are also applied to CO_2 capture by microalgae cultivation. For example, a semi-continuous technique in outdoor bag photobioreactors was employed for *Chlorococum* sp. cultivation. After harvesting the culture on a daily basis, 20% (v/v) dilution was carried out with a fresh medium to maintain the culture volume. The left over culture after periodic harvesting served as inoculums for culture replenishment, with enormous water conservation [18].

4. Dewatering, extraction and transesterification

4.1. Dewatering

Microalgal culture dewatering is a major obstruction to industrial-scale processing of microalgae for biofuel and other high-value biochemicals production due to the very dilute nature of harvested microalgal cultures, resulting in high energy con-

sumption required for dewatering. This also causes much CO_2 emission during dewatering, thus making microalgae-based products less economically attractive [95]. Currently, there is no superior method for dewatering microalgae culture. The common techniques include centrifugation, flocculation, filtration (such as high pressure filtration and tangential flow filtration) and gravity sedimentation. The choice of dewatering technique is dependent on the microalgae species and the desired product quality. Centrifugation is seen as the most efficient biomass recovery technique. However the energy and capital costs associated with it, especially for large-volume processing, are unappealing. Flocculation and filtration is potentially an efficient technique that is currently applied in industries [95,96].

4.2. Pre-treatment and extraction

Pre-treatment of concentrated microalgal culture, such as drying or cell disruption, has been shown to aid lipid or protein extraction [97]. An advantage of processing dried biomass is better percolation of solvents or fluids through the cell to improve extraction efficiency. However, drying is not considered as an economically viable option for biomass production because of the high-energy requirements [98,99]. Cell rupture techniques of the harvested biomass, which could aid extraction by avoiding or reducing the use of solvents include mechanical, chemical and enzymatic treatments, and these have been described in publications as applied to oilseeds and microalgae [98,100–102]. Due to the high energy consumption of mechanical rupturing, environmental issues associated with harsh and toxic chemicals for cell lysis and the slow rupturing kinetics of enzymatic exposure, none of these methods have been adopted for microalgae on a large-scale [99]. After pre-treatment, lipids and proteins can be recovered from microalgal biomass by means of physical processes (such as cold press) or extraction or both [103]. Non-polar organic solvents such as n-hexane, acetone, and chloroform are effective solvents for non-polar products [104]. However, in the last decades, concerted efforts have been made to increase extraction efficiency, and to reduce the use of toxic and polluting organic solvents through the development of supercritical fluid extraction [105]. Supercritical fluid extraction, though a benign technique, has a high investment and a high operating cost due to energy consumption during fluid compression.

4.3. Transesterification

Saponifiable lipids such as triacylglycerols (TAG) and phospholipids are converted to fatty acid methyl esters by the addition of excess methanol and catalyst, in a reaction known as transesterification. Transesterification involves the cleaving of an ester bond by an alcohol under either an alkali or acid catalysis. Alkaline catalysts, such as sodium methoxide, or sodium/potassium hydroxide in methanol, transesterify complex lipids quickly and at lower temperatures than required by acid catalysts, but they do not esterify free fatty acids. Acid catalysts, such as hydrochloric or sulphuric acid in methanol or boron trifluoride (BF_3) methanol, require heating and longer reaction times than basic catalysts, but can transesterify complex lipids as well as esterify free fatty acids [106,107].

Instead of improving extraction methods, some investigators have eliminated extraction completely by transesterifying lipids in situ. Direct transesterification was first successfully performed in 1963 [108]. Since then, it has been verified by numerous researchers in a variety of tissues, as a simple and rapid method of quantifying fatty acids by combining extraction and transesterification into one step. Diverse methods have been used for direct transesterification, but most involve the addition of an organic solvent,

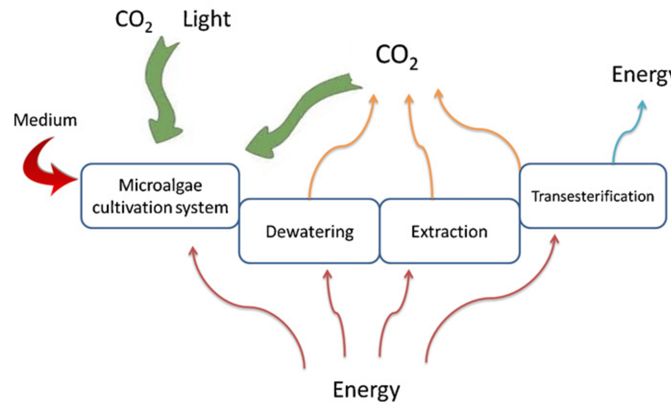


Fig. 2. CO_2 fixation and energy generation for a microalgal biodiesel production system.

methanol, catalyst and heat to a small amount of dried sample [106]. However, in recent years, lipase, as a biocatalyst, has been employed in direct transesterification, to offer little adverse effect on the environment [109]. A further advancement in the area of biocatalysts for direct transesterification is immobilized-biocatalyst, where the whole cell, lipase included, is used as a recycled catalyst. This can considerably decrease the biocatalyst cost, since separation and immobilization is realized spontaneously during the process [109–111].

5. CO_2 fixation ability assessment

In this CO_2 mitigation assessment, an open pond cultivation system of *C. vulgaris* based on a standard design was employed [112,113]. The cultivation conditions were selected to simulate those of Scragg et al. [65]. As shown in Fig. 2 which is drawn by the authors, CO_2 is supplied from external sources such as air, flue gases from boilers, furnaces, or power plants for microalgae growth. After the growth of microalgae to the harvest concentration, culture dewatering is performed by flocculation with filtration through a chamber filter press or centrifugation in a self-cleaning plate separator centrifuge, followed by drying in a natural gas fired dryer. The microalgae are dried to 9 wt% moisture, being the required moisture content for hexane extraction [95]. After the harvesting, dewatering and drying steps, lipids present in the microalgae are extracted by n-hexane. Hexane extraction and transesterification of resulting microalgal oil into biodiesel were modelled based on a previous soybean biodiesel LCA [113,114]. Growth, harvest, dewatering/drying, oil extraction from algae and transesterification are assumed to be performed at the same site. 24 kg of biodiesel combustion produces 1000 MJ energy [5]. 0.584 kg (1.341 pounds) CO_2 emission is made by electrical machine consuming 1 kWh electricity. Table 3 shows the assessment of energy generation and CO_2 fixation ability of microalgal biodiesel production system.From Table 3, dewatering consumes more than 75% energy required for the entire oil production system. Microalgal culture dewatering with flocculation and filtration for the production of 24 kg biodiesel generates 85.56 MJ of energy (energy input subtracted) with 50.02 kg net CO_2 fixation [113] and a cost of 40–60 US dollars [99]. There are obvious differences with different dewatering techniques. A 1000 m^2 microalgae cultivating area (0.5 m depth) with growth rate 30 g/m^2 d, 30% microalgae lipid content, and harvesting, extraction and transesterification efficiencies 90%, will generate 11,000 kg biomass, 3300 kg biodiesel per year and a net CO_2 fixation of 7000 kg. Hence the average biodiesel productivity is 3.3 kg/m^2 per year and the net CO_2 fixation capacity 7 kg/m^2 per year. Considering energy generation combined with CO_2 fix-

Table 3
Energy input and CO_2 fixation of a microalgal biodiesel production process [95,113].[a]

Processes	Growth	Dewatering	Separation	Diesel conversion	Total balance
Flocculation and filtration primary dewatering					
Energy generate (MJ)	−15.43	−728.82	−165.03	−36.02	85.56
CO_2 fixation (kg)	120	−60.47	−6.33	−3.18	50.02
Filter press primary dewatering					
Energy generate (MJ)	−15.43	−2915.27	−165.03	−36.02	−2131.75
CO_2 fixation (kg)	120	−241.87	−6.33	−3.18	−135.93
Centrifuge primary dewatering					
Energy generate (MJ)	−15.43	−5743.32	−165.03	−36.02	−4959.80
CO_2 fixation (kg)	120	−398.48	−6.33	−3.18	−287.99

[a] Basis: to produce 1000 MJ biodiesel energy "−" states for negative.

ation capacity, as well as growth rate, biofuels from microalgal biomass are much more preferable compared to biofuels generated from cellulosic or microbial biomass [115,116]. However, due to the dilute nature of harvested microalgal culture, much more research work on cultivation, energy-efficient dewatering and lipid extraction techniques should be focus.

6. Conclusions

Increase in atmospheric CO_2 concentration and the depletion of mineral oil reserves require the rapid development of carbon-neutral renewable alternatives. CO_2 fixation by microalgae provides a promising alternative for mitigation of CO_2, feedstock for biofuels and other high-value products. Simultaneously, wastewater can be treated or demineralised using this system. This could thus present a sustainable process integration of CO_2 capture, wastewater treatment and biofuel production. At present, there are few examples of large-scale continuous microalgae-based CO_2 capturing system [117,118]. However, laboratory and pilot plant studies suggest that capturing CO_2 by microalgae is a potentially viable strategy for mitigating CO_2 emissions from anthropogenic sources. The long-term prognosis for large-scale carbon capture using microalgae will depend on the passage of legislation promoting carbon-capture technologies and the improved economics of microalgal pond systems. Significantly, life cycle and economic assessments suggest that the costs of capturing carbon and producing liquid biofuels from microalgae may approach the cost of producing petroleum-based fuels in the next decades, and the microalgal biofuels will be one of the main biofuel products [17,71,98,119].

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